

INSIGHTS FROM MODEL SYSTEMS Genes That Regulate Eosinophilic Inflammation

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Eosinophils and neutrophils may be considered close cousins, because of their shared bone-marrow origin, circulation in the blood stream, and egress at tissue sites of inflammation. Under the influence of distinct cytokines, both eosinophils and neutrophils differentiate in the bone marrow and enter the bloodstream. Both cell types interact with the surface of inflamed endothelium, diapedese between endothelial cells, and migrate through the extracellular matrix at tissue sites of inflammation, where they eventually release their stores of inflammatory mediators.

Although the trafficking patterns of neutrophils and eosinophils from bone marrow to tissue sites of inflammation have much in common, there are distinct differences in their association with different disease states. For example, tissue recruitment of neutrophils is prominent at sites of bacterial infection, whereas tissue recruitment of eosinophils is prominent at sites of parasitic infection and allergen challenge. The distinct patterns of localization of these closely related circulating leukocytes suggest that either the nature of the inflammatory stimulus (i.e., bacteria, parasite, or allergen); the profile of cytokines, chemokines, and mediators released in response to the inflammatory stimulus; or the profile of adhesion receptors and chemokine receptors expressed by the eosinophil as compared with the neutrophil contributes to their different tissue localization in response to a particular inflammatory stimulus. An improved understanding of the factors responsible for the localization of eosinophils at sites of allergic inflammation may allow for therapies for allergic inflammation that selectively inhibit eosinophil tissue recruitment but that neither influence neutrophil tissue recruitment nor compromise the host's ability to defend against bacterial infection.

Asthma, a common respiratory disease affecting ~3%

Received May 24, 1999; accepted for publication June 22, 1999; electronically published July 15, 1999.

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0002-9297/99/6502-0005\$02.00

of the U.S. population, is a disorder with a strong hereditary component but complex inheritance. The characteristic features of asthma—airway inflammation, bronchial hyperresponsiveness, and reversible airway obstruction—arise in part through the activity of eosinophil derived proinflammatory mediators, including a stored granule component—major basic protein—as well as cytokines and lipid mediators, which can be synthesized at the site of inflammation (Broide et al. 1991, 1992; Weller 1997; Rothenberg 1998). These factors contribute to airway smooth-muscle contraction, mucus secretion, and denudation of the airway epithelium, all of which are hallmarks of asthma. The importance of the eosinophil in asthma and other allergic responses has prompted considerable interest in the various stages of eosinophil development, motility, and activation. Here we discuss genetic and physiological studies that have begun to clarify these events. At several points we compare the properties of eosinophils with those of neutrophils, because of the clinical interest in therapies for allergic asthma that might selectively suppress eosinophils while sparing neutrophils.

Eosinophil Differentiation: The Role of IL-5

Interleukin (IL)-5 plays at least two roles in the generation of blood-borne eosinophils. First, it stimulates bone marrow CD34⁺ progenitor cells, the precursors of both eosinophils and neutrophils, to differentiate into eosinophils. Second, it induces the mobilization of eosinophils from the bone marrow into the bloodstream (Palframan et al. 1998). The release of mature eosinophils from the bone marrow is a multistep process involving the release of eosinophils from bone-marrow stromal cells and extracellular matrix (ECM), the migration of eosinophils across the bone-marrow sinus endothelium, and the release of eosinophils from the luminal surface of the endothelium (Palframan et al. 1998). Once released, eosinophils traffic freely through the bloodstream, until their surface receptors encounter specific counterreceptor proteins that are expressed, by the local endothelial cells, at sites of allergic inflammation. Once bound firmly to such an endothelial-cell

surface, eosinophils are induced by inflammatory chemokines to migrate into tissue sites.

The recruitment of eosinophils into the airway can be reproduced experimentally in mouse and human models of allergen-induced asthma. In people sensitized to an allergen such as house dust-mite allergen, inhalation challenge with that allergen induces a significant airway eosinophilic and IL-5 response 24 h later (Broide et al. 1992). IL-5-deficient mice are unable to develop eosinophilia after allergen sensitization and challenge (Foster et al. 1996). Moreover, studies that use DNA immunization to inhibit allergic inflammation (either because the DNA encodes an allergen or because the noncoding DNA contains a CpG sequence that serves as an adjuvant that stimulates T-helper 1 cells) recently have confirmed that DNA-mediated inhibition of IL-5 can suppress eosinophilic inflammation and bronchial hyperresponsiveness in a mouse model of asthma (Hsu et al. 1996; Broide et al. 1997, 1998b; Kline et al. 1998).

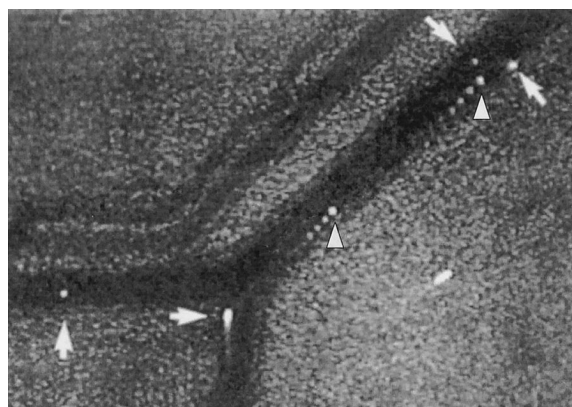
In humans, the *IL5* gene is located on 5q31, and some groups have reported 5q associations with asthma (Daniels et al. 1996), autosomal dominant familial eosinophilia (Rioux et al. 1998), and circulating eosinophil levels (Martinez et al. 1998). Baranger et al. (1994) also have identified chromosomal translocations involving 5q31-33, in malignancies associated with eosinophilia. However, other candidate genes on chromosome 5q—such as the genes for the β_2 adrenergic receptor, the corticosteroid receptor, GM-CSF, IL-3, and IL-9—have also been linked to asthma. Four genomewide screens (Daniels et al. 1996; Collaborative Study of the Genetics of Asthma 1997; Ober et al. 1997; Stine et al. 1998) have identified a common set of loci (at 5q, 6p, 11q, 12q, 13q, and 16q) with probable linkage to susceptibility to asthma, bronchial hyperresponsiveness, or allergic inflammation. Despite this overall agreement, none of the loci were identified by all four studies, and several other loci were identified that may affect the asthma phenotype in particular populations. These studies underscore the complex relationship of genes, the environment, and the development of asthma in different populations.

Regulation of Adhesive Interactions in Circulating Eosinophils and Neutrophils

After inhalation of allergen, resident mucosal mast cells and macrophages secrete cytokines, including IL-1, IL-4, and tumor-necrosis factor (see Ruuls and Sedgwick 1999 [in this issue]). These cytokines act on neighboring endothelial cells to up-regulate their expression of adhesion molecules. Eosinophils in blood vessels may be visualized *in vivo* by intravital videomicroscopy (see sidebar). This research method demonstrates that eosinophils interact with inflamed endothelium first by rolling

Watching a Drifter Change Its Ways

Classic studies of the interactions between leukocytes and endothelial cells invariably have probed the binding of these cell types in a static setting. Endothelial cells, plated as a monolayer, were treated with stimulatory cytokines, and cells such as eosinophils or neutrophils were introduced and allowed to bind for a period of time. Such assays are limited in several respects, perhaps most importantly in that they do not take into account the effect that blood circulation has on leukocyte adhesion. Intravital videomicroscopy provides a complementary *in vivo* technique that allows one to visualize (and to record on videotape) leukocyte trafficking as blood flows in the living vasculature of rabbits, mice, or rats. We have examined the progress of eosinophils through blood vessels in the skin, mesenteric circulation, and lung, and others have used similar techniques to study cell migration in lymph nodes.



Intravital microscopy studies can be performed either on mice that lack specific adhesion molecules or on wild-type animals in which adhesion-blocking antibodies are introduced intravenously. Both approaches allow one to test the role of the adhesion molecule in cell movement within the vessel. This photomicrograph shows fluorescently labeled human eosinophils rolling and adhering to rabbit postcapillary venules. The eosinophils, purified from peripheral blood, are injected intravenously into an anesthetized rabbit. The animal's mesenteric circulation is exteriorized so that it can be stimulated with either an allergen or a specific cytokine such as IL-1 (to mimic aspects of allergic inflammation) and be examined under stroboscopic illumination using a videomicroscope. In the unstimulated mesentery, eosinophils rarely interact with the endothelium but pass freely through the circulation. In contrast, in the IL-1-stimulated mesentery shown here, many of the eosinophils roll slowly along the surface of the endothelium. Blood flow is from the lower left to upper right. Noninteracting eosinophils (arrowheads) are captured in several discrete positions by successive flashes of the stroboscope. In contrast, rolling eosinophils proceed more slowly along the endothelial surface and produce a cometlike trail in the image. As discussed in the main text, neutralizing antibodies to either L-selectin or α_4 integrins—eosinophil surface proteins that bind specifically to proteins on the surface of the activated endothelium—significantly reduce eosinophil rolling.

The electronic version of the *Journal* provides a videomicrograph of eosinophil rolling; see the *Journal's* Website [<http://www.journals.uchicago.edu/AJHG/journal>].

along their surface in the direction of blood flow and then by adhering tightly to a single endothelial location before transmigrating across the vessel and into the abluminal tissue (Sriramarao et al. 1994, 1996). The first step of the eosinophil/endothelial interaction is a low-affinity tethering interaction between receptors expressed by the fast-flowing eosinophil in the blood stream and counterreceptors expressed by inflamed endothelium. This low-affinity interaction results in transient adhesion and detachment, permitting the eosinophil to roll along the endothelial surface sufficiently slowly that it can respond to local biochemical cues that promote firm adhesion. For example, the chemokine eotaxin, which binds to the receptor protein CCR3 on eosinophils, induces the eosinophil to arrest on endothelium (Kitayama et al. 1998).

Considerable research has focused on understanding the selective adhesion pathways that recruit eosinophils to sites of allergic inflammation but that do not act on neutrophils. As depicted in figures 1 and 2, the two cell types share some adhesion pathways, but other molecular interactions appear to be unique to eosinophils. Both eosinophils and neutrophils express L-selectin and P-selectin glycoprotein-1 (PSGL-1), and both cell types use these cell-surface receptors to bind loosely (and, hence, to roll) on counterreceptors expressed by inflamed endothelium. Eosinophils, however, also use $\alpha_4\beta_1$ integrin and $\alpha_4\beta_7$ integrin (receptors that are not expressed on neutrophils) to roll on the endothelial surface protein VCAM-1 (Sriramarao et al. 1994).

The importance of selectin adhesion molecules to neutrophil recruitment at sites of infection is suggested from studies of patients with the congenital immunodeficiency known as "leukocyte adhesion deficiency syndrome 2" (LAD-2). Neutrophils from patients with LAD-2 lack a critical enzyme in the biosynthesis of sialyl-Lewis X (SLE^X), a carbohydrate that binds specifically to E- and P-selectins (Phillips et al. 1995). Because they lack SLE^X , neutrophils from patients with LAD-2 bind minimally or not at all to E-selectin or P-selectin expressed by endothelium, and they fail at the first step of tissue recruitment to sites of bacterial infection, the induction of rolling on the inflamed endothelial surface.

The endothelial-cell surface expresses adhesion counterreceptors that bind either to both neutrophils and eosinophils or selectively to a particular subset of circulating leukocytes, to permit leukocyte rolling (see figs. 1A and 2A). For example, both eosinophils and neutrophils roll efficiently on P-selectin, but only neutrophils can interact in this manner with E-selectin (Sriramarao et al. 1996). Thus, in the first step of leukocyte/endothelial interactions, there are both eosinophil-specific interactions ($\alpha_4\beta_1$ or $\alpha_4\beta_7$ integrins with the endothelial counterreceptor VCAM-1), neutrophil-specific interactions (SLE^X with the endothelial counterreceptors E- and P-selectin), and common eosinophil and neutrophil interactions with endothelial counterreceptors (PSGL-1 binding to P-selectin or L-selectin binding to potential counterreceptors including GlyCAM-1, CD34, or MAdCAM-1).

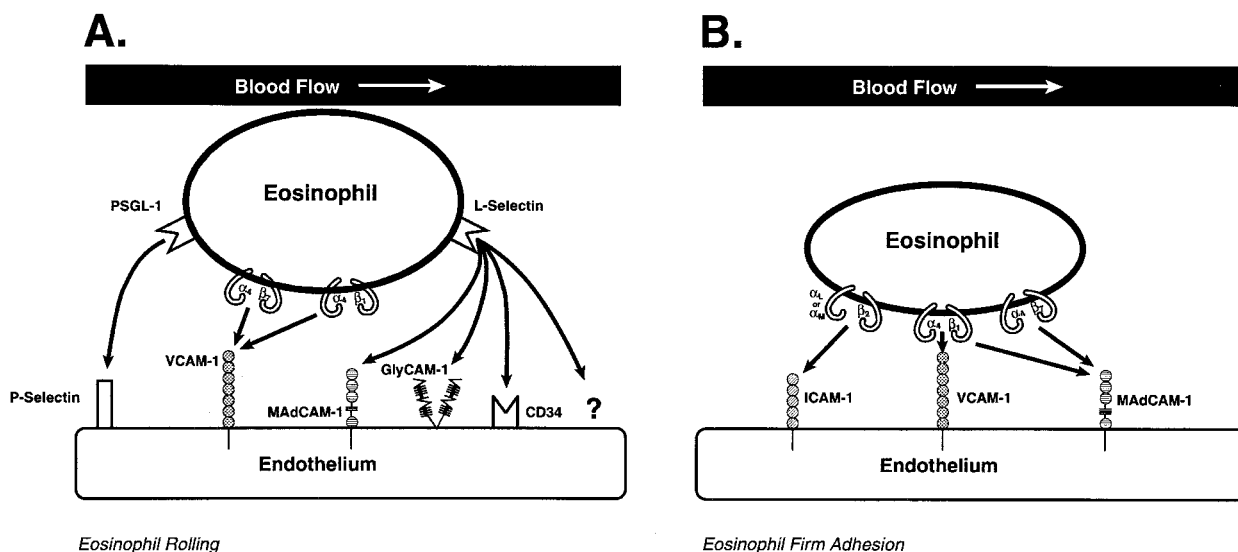


Figure 1 Adhesion receptors mediating eosinophil rolling and firm adhesion to endothelium. Eosinophils use both integrin-mediated and selectin-mediated adhesion for their initial rolling interaction with the endothelium (A), and they bind firmly to endothelium by using several classes of integrins (B). Eosinophils differ from neutrophils in that they utilize $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins to both roll and firmly adhere to VCAM-1 expressed by endothelium. Their induction of firm adhesion depends in part on changes in the intrinsic affinity of these integrins for their endothelial counterreceptors, the cell-adhesion molecules VCAM-1 and MAdCAM-1.

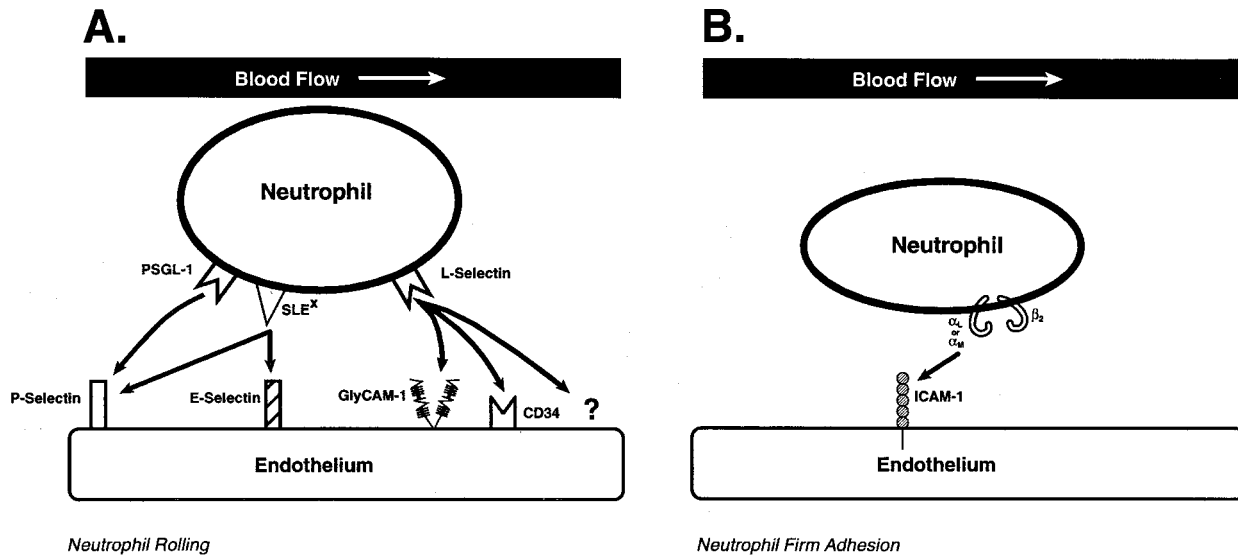


Figure 2 Adhesion receptors mediating neutrophil rolling and firm adhesion to endothelium. Neutrophils rely on a different set of receptors for their rolling (A) and their firm-adhesion (B) interactions with the endothelial surface. Congenital immunodeficiencies can arise from defects in expression of neutrophil-adhesion receptors. Neutrophils from patients with LAD-1 do not express β_2 integrins and thus cannot firmly adhere to ICAM-1 expressed by endothelium. Because they lack SLE^x , neutrophils from patients with LAD-2 bind minimally and do not roll on P-selectin or E-selectin.

Firm adhesion, which prevents leukocyte detachment from the endothelium despite the effects of blood flow, is also controlled quite differently in eosinophils and neutrophils (figs. 1B and 2B). Eosinophils bind tightly both to ICAM-1 and to VCAM-1, whereas neutrophils adhere tightly only to ICAM-1. Eosinophils express at least three different integrin adhesion receptors— $\alpha_4\beta_1$, $\alpha_4\beta_7$, and the recently identified $\alpha_d\beta_2$ —that serve as receptors for endothelial VCAM-1 (Grayson et al. 1998). In addition to the β_1 integrins, eosinophils and neutrophils express three types of β_2 integrins—collectively known as “CD11/CD18”—that interact with ICAM-1. All three types of β_2 integrin receptors are heterodimeric transmembrane proteins containing a unique α chain and a shared β_2 chain—that is, $\alpha_1\beta_2$ (also known as “CD11a/CD18” or “LFA-1”), $\alpha_M\beta_2$ (also known as “CD11b/CD18” or “Mac-1”), and $\alpha_X\beta_2$ (also known as “p150,95” or “CD11c/CD18”). Neutrophils, like eosinophils, express CD11/CD18 β_2 integrins and can bind tightly to ICAM-1, but they lack $\alpha_4\beta_1$ and $\alpha_4\beta_7$ and thus cannot bind to VCAM-1. Interestingly, in the congenital immunodeficiency syndrome LAD-1, patients suffer from recurrent bacterial infections and exhibit high peripheral-blood neutrophil-cell counts, but their tissue neutrophils do not home to sites of infection. The molecular defect in these individuals has been identified in the *ITGB2* gene, which encodes the common β_2 integrin subunit. Neutrophils from patients with LAD-1 do not express β_2 integrins and thus cannot bind firmly to ICAM-1 expressed by inflamed endothelium. Thus, neu-

trophils can enter the bloodstream from the bone marrow but cannot exit at sites of infection. Eosinophils (cells that, like the neutrophils in such patients, lack β_2 integrins) from patients with LAD-1 can bind normally to endothelial VCAM-1, using $\alpha_4\beta_1$ and $\alpha_4\beta_7$, so they can enter sites of inflammation even though they fail to interact with ICAM-1.

Targeted ablation of mouse genes for these adhesion receptors and the use of antibodies that block their adhesion function confirm the importance of these adhesion receptors in eosinophil recruitment. Using intravital videomicroscopy (see sidebar), we have demonstrated that eosinophil rolling and firm adhesion to endothelium, as well as eosinophil tissue recruitment, are significantly reduced in both P-selectin-deficient mice and ICAM-1-deficient mice (Broide et al. 1998a). The significance of the VCAM-1 adhesion pathway for eosinophil recruitment is less clear and may vary in different species (Abraham et al. 1994; Nakajima et al. 1994; Henderson et al. 1997). Since eosinophils can use ICAM-1-dependent pathways to bypass the need for VCAM-1, it is not surprising that inhibition of VCAM-1 does not completely abolish eosinophil recruitment. However, neutralizing antibodies to α_4 integrins improve airway function in some animal models of asthma in which eosinophil migration is only partially inhibited (Abraham et al. 1994; Henderson et al. 1997). This effect may be explained by the direct effects of the anti- α_4 antibody on proinflammatory responses of eosinophils or other cell types expressing α_4 receptors, as opposed to effects

on adhesion. It is known that the portion of the alternatively spliced CS-1 exon of the ECM molecule fibronectin binds $\alpha_4\beta_1$. Ligation of this integrin activates eosinophil expression of the cytokine GM-CSF, and the blocking antibody to α_4 inhibits this cytokine induction. Hence, treatment with this anti- α_4 antibody may affect proinflammatory cytokine expression, as well as interfere with eosinophil adhesion to the endothelium.

The ability of integrins such as $\alpha_4\beta_1$ to mediate both the early, rolling interaction with the endothelial surface and the later, tight-adhesion function is consistent with cell-culture studies in which the functional state of this integrin is switched from a low-affinity to a high-affinity state by cellular agonists. Sung et al. (1997), using a single-cell micropipette adhesion assay, have demonstrated that cytokines such as GM-CSF increase the affinity that eosinophil $\alpha_4\beta_1$ has for both VCAM-1 and CS-1. This change is not associated with altered integrin expression level or surface cellular distribution, but it appears to represent an increase in receptor avidity. GM-CSF also affects β_2 -integrin-mediated interactions in eosinophils, by up-regulating β_2 expression, and it induces the shedding of L-selectin from the surface of the eosinophil. Thus, GM-CSF and, perhaps, other cytokines that are released at sites of allergic inflammation appear to control the adhesive properties of eosinophils, permitting them to stop rolling over the endothelium and to remain bound to it tightly in place.

Roles of Chemokines in Eosinophil Chemotaxis into Tissues

Differential chemoattractant responses by eosinophils and neutrophils to individual chemokines play an important role in the selective recruitment of endothelial cell-adherent eosinophils and neutrophils to sites of inflammation. Chemokines have been classified into subsets based on the position of the conserved cysteine residues in their 8–10-kD amino acid sequence. CC chemokines have two juxtaposed cysteine residues, whereas CXC chemokines have an additional amino acid (X) separating the two cysteine residues. As a rule, neutrophils migrate in response to chemokines of the CXC class, whereas eosinophils respond to CC chemokines. Several CC chemokines important to eosinophil recruitment are expressed at sites of allergic inflammation after allergen challenge, and neutralizing antibodies to CC chemokines or chemokine receptors, as well as genetic ablation of CC chemokines, block eosinophil recruitment. The chemokine eotaxin has generated considerable interest because of its specific effect on eosinophils. Eosinophil recruitment is blocked early after allergen challenge in eotaxin-deficient mice (Rothenberg et al. 1997), but not at later points in the process, suggesting that other chemokines can function in this

pathway at later time points. Indeed, Gonzalo et al. (1998) recently have shown that chemokines such as RANTES, MCP-5, and MIP1 α are also important in eosinophil tissue recruitment.

The selectivity of the eosinophil response to a particular chemokine is due to the chemokine-receptor profile expressed by eosinophils. Eosinophils predominantly express the CCR3 receptor, to which eotaxin binds, with lower levels of CCR1 (Kitaura et al. 1996; Ponath et al. 1996). Because RANTES and MCP-3 also bind to CCR3, this receptor appears to be an attractive therapeutic target for inhibition of eosinophil recruitment, particularly in light of the finding that neutralizing antibodies to CCR3 block the binding of eotaxin, RANTES, and MCP-2, -3, and -4 to human eosinophils (Heath et al. 1997; Kitayama et al. 1998).

Acknowledgments

The authors wish to thank Lanesha Hill for secretarial support during the preparation of the manuscript. This work was supported by National Institutes of Health grants AI33977 and AI38425 (both to D.H.B.) and AI35796 (to P.S.).

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